PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **307th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University of Liverpool, Liverpool* 3, *on Friday,* 18 *April* 1952 *when the following papers were read.*

COMMUNICATIONS

A Vitamin B_{12}-binding Factor in Sow's Milk.

By MARGARET E. GREGORY, J. E. FORD and S. K. KON. (*National Institute for Research in Dairying, University of Reading*)

A sample of cow's milk, prepared for assay by warming in the presence of a trace of sodium cyanide, contained 0·004 μg. vitamin B_{12}/ml. by the *Lactobacillus leichmannii* 4797 assay. Vitamin B_{12} added to the milk before extraction was quantitatively recovered. However, *Lb. leichmannii* did not respond to a similar extract of sow's milk or to vitamin B_{12} added to the extract up to a concentration of 0·05 μg./ml. whole milk, but responded to additions over and above that amount.

Vitamin B_{12} was largely (92 %) recovered in the ultrafiltrate from cow's milk to which 0·04 μg. vitamin B_{12}/ml. had been added, whereas none could be detected in the ultrafiltrate from sow's milk similarly treated. Furthermore, vitamin B_{12}, added at a rate of 0·05 μg./ml. whole milk, could not be dialysed from sow's milk, but 90 % dialysed from cow's milk. Therefore, it appears that vitamin B_{12}, added to sow's milk, is bound and microbiologically inactivated. This behaviour of sow's milk is similar to that reported by Bird & Hoevet (1951) for an intrinsic factor concentrate. Since the vitamin B_{12} naturally present in cow's milk was not ultrafiltrable, it must also be 'bound', but in a form which, after being heated with the assay medium, is available to micro-organisms.

The vitamin B_{12}-binding component of sow's milk survived heating at 100° for 30 min. It was destroyed by autoclaving at pH 12, but not at pH 7 unless a trace of sodium cyanide was present. It was not ultrafiltrable and was inactivated by papain digestion.

Fractionation of the soluble whey proteins with ammonium sulphate, according to the procedure of Prusoff, Meacham, Heinle & Welch (1950) for the isolation of the intrinsic factor from powdered hog stomach, indicated that the vitamin B_{12}-binding activity was associated with the fraction precipitated by 35–55 % ammonium sulphate. Sow's milk casein had no binding activity.

Further work on the isolation and characterization of this binding factor is in progress.

We wish to thank Dr R. Braude and Mr K. G. Mitchell for samples of sow's milk.

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Determination of Sulphate in the Study of Sulphatases.

By K. S. DODGSON and B. SPENCER. (*Physiology Institute, Newport Road, Cardiff*)

Studies on sulphatases have been hindered in the past by the lack of suitable methods for the determination of small amounts of sulphate under varying experimental conditions. It appeared to us that the micro-benzidine method might be adapted for this purpose. Various modifications of this method have been described (e.g. Tanaka, 1938; Power & Wakefield, 1938; Eichelberger, Brower & Roma, 1951), but all demand rigid adherence to the prescribed conditions. Considerable research has been necessary in order to establish techniques suitable for enzyme studies.

Briefly, the incubated enzyme-substrate mixture is deproteinized with ethanol, the acidity of the protein-free solution is adjusted to within certain critical limits with trichloroacetic acid, and sulphate is precipitated as benzidine sulphate with an ethanolic solution of purified benzidine. The benzidine sulphate is separated and determined colorimetrically after diazotization and coupling with thymol. In this and other modifications which we have tested, sulphate is not precipitated quantitatively in amounts less than 10 μg., consequently enzyme control determinations may be low. In

practice this difficulty is met by ensuring that the amount of sulphate in the control is always of the order of 15 μg. In experiments with marine organisms this amount of sulphate is normally encountered, but with mammalian tissues it is necessary to add sulphate (preferably in the trichloroacetic acid) to tests and controls immediately before addition of benzidine.

With this method we can estimate differences between enzyme test and control of 10–120 μg. with an accuracy of $\pm$ 5 %; while the method is useful for measuring even smaller differences. Added sulphate can be recovered quantitatively from various tissue homogenates of the rat, cat, dog and many marine organisms and seaweeds after incubation periods of up to 3 hr. and in the presence of acetate buffers of varying molarity and pH. Recoveries are not affected by K, Na, Mg, Cl, F, CN and PO_4 ions at concentrations of 0·01 M, but results are low in the presence of similar concentrations of Ca, Ba and Fe.

We have checked this method against the spectrophotometric assay method of Dodgson, Spencer & Thomas (1952) and find that the arylsulphatase of various rat organs releases *p*-hydroxyacetophenone and inorganic sulphate from potassium *p*-acetylphenylsulphate in molecular proportions.

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The Action of Crystalline β-Amylase on Some Glycogens. By D. J. Manners. (*Biochemical Laboratory, University of Cambridge*)

β-Amylolysis of amylopectins and glycogens is now generally regarded as a stepwise hydrolytic liberation of maltose from the exterior chains. The amount of maltose thus formed is determined by the position of the branching point of the unit chain, and hence by the exterior chain length.

Glycogens from liver (rabbit, foetal sheep, cat), muscle (rabbit, horse, man), and invertebrates (*Mytilus edulis* I, *Ascaris lumbricoides*) of unit chain lengths 11–13 radicals (potassium periodate method, Halsall, Hirst & Jones (1947) as modified by Bell & Manners, 1952) were treated to completion with crystalline sweet potato β-amylase, kindly supplied by Prof. C. S. Hanes, F.R.S. The resulting conversions to maltose (41–49 %) corresponded to β-limit dextrins of chain lengths 6–7.

Three other glycogens have been examined: (*a*) *Mytilus edulis* II (unit chain length 16 by periodate, and 18 by methylation (Bell, 1936)) gave 47 % conversion to maltose corresponding to a β-limit dextrin of 9–10 glucose radicals; (*b*) *Mytilus edulis* III (unit chain length approx. 5 by periodate, cf. Halsall *et al.* 1947) gave 43 % conversion to maltose, corresponding to a β-limit dextrin of three glucose radicals; (*c*) *Helix pomatia* (unit chain length 7 by periodate; cf. Baldwin & Bell (1940), who found a chain length of 12, by methylation, for a different sample) gave 37 % conversion to maltose, corresponding to a β-limit dextrin of 4 glucose radicals. Control experiments showed that the β-amylase was free from maltase, α-amylase and *Z*-enzyme.

It is interesting to compare the above β-amylolysis of 12-unit glycogens with those of an 18-unit rabbit-liver glycogen. Halsall, Hirst, Hough & Jones (1949) found that this material gave 53 % maltose on β-amylolysis, and a β-limit dextrin of 9 glucose radicals. Amylopectins (unit chain length 19–26, cf. Bell, 1947) give β-amylolysis limits of 50–62 %, and β-limit dextrins of chain length 11–13.

The present study supports, chemically, the suggestion of Barker, Bourne & Stacey (1950) 'that there is no clear-cut distinction in nature between glycogen and amylopectin'. Between the extremes, 12-unit glycogens and 25-unit amylopectins, lie a group of polysaccharides of intermediate properties, including 18-unit glycogens, and certain bacterial and protozoal polysaccharides.

I wish to thank Dr D. J. Bell for the glycogen samples, and for valuable advice and encouragement.

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The Metabolism of [1-^{14}C]-Benzene. By D. V. PARKE and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*)

Previous work from this laboratory on the metabolism of benzene in the rabbit had shown that about 60 % of the benzene fed could be accounted for in various ways. The acquisition of benzene containing one carbon atom marked with ^{14}C has now enabled us to account for most of the dose of benzene administered and to estimate the amounts of the various metabolites excreted. Thus in a rabbit which had received 1 g. of benzene containing 0·1 mc. of radioactivity, 46 % of the benzene appeared in the expired air as benzene in 30 hr., 35 % in the urine as metabolites and 16 % remained in the tissues as metabolites after 2 days (total, 97 %). In this particular experiment the percentage of the dose excreted as various metabolites in 2 days, determined by isotope dilution, was as follows: phenol, 16·7; quinol, 6·2; catechol, 1·2; hydroxyquinol, 0·35; *trans-trans*-muconic acid, 1·8; phenylmercapturic acid, 0·7 %. By the same technique it was found that the following substances were not urinary metabolites of benzene: resorcinol, pyrogallol, diphenyl, *p*-diphenylylglucuronide, *cis-cis*- and *cis-trans*-muconic acids, and oxalic, succinic, adipic and acetic acids. In another experiment with 0·88 g. benzene containing 4 μc. of ^{14}C, radioactive metabolites were being excreted for 7 days after dosing, 29·7 % of the dose appearing on the first day and 0·4 % on the seventh day. In this case 41·3 % of the benzene was excreted in the urine as metabolites in 7 days, and 34 % in the expired air in 2 days. A 2-day urine in this case contained phenol, 23·7; quinol, 4·8; catechol, 2·1; hydroxyquinol, nil; *trans-trans*-muconic acid, 1·0 % of the dose. These items account for 31·6 % of the dose in 2 days, and this compares well with the figure 34·8 % as the total radioactivity of the 2-day urine.

These and other results were discussed.

A Visual Matching Technique for Paper Electrophoresis of Serum Proteins. By A. L. LATNER and M. W. RICHARDSON. (*Department of Pathology, Medical School, King's College, Newcastle-upon-Tyne*)

This method is suitable for most clinical purposes and requires no photoelectric scanning device.

A pencil line is drawn perpendicular to the long edge across a strip of Whatman no. 1 filter paper 37 × 2 cm. at a distance of 2 cm. from the mid-line. On either side of this original line and 2 mm. from it two further lines are drawn.

Using a micro-pipette or brush, serum is applied along the central line at such a rate that it just spreads to the outer lines. In this way a band of serum 4 mm. wide is obtained across the paper.

The process is repeated on four other strips of paper using 8/10, 6/10, 4/10 and 2/10 dilutions of the serum in normal saline. The serum bands are dried, separated electrophoretically and subsequently stained together according to the technique already demonstrated by one of us (Latner, 1952), although the apparatus used was that of Flynn & de Mayo (1951). The albumin is made to pass down the paper and not over the glass rod.

The albumin bands were originally given numbers 10, 8, 6, 4 and 2, according to the serum dilution. The stained protein bands on the strip made with whole serum are visually matched against each albumin band on the other strips using Ilford Filter no. 204. A number is thus assigned to each of the bands of the whole serum and the percentage of each is calculated:

$$\% = \frac{\text{Band number} \times 100}{\text{Sum of band numbers}}.$$

The band of γ-globulin has approximately twice the other band widths. It is therefore read as if composed of two bands.

It soon became obvious that the spread of a protein solution such as serum is dependent on the protein concentration. This meant that the albumin bands were not in the proportion 10, 8, 6, 4 and 2 and corrections had to be applied. These were determined by studying the area of spread of fixed volumes of varying dilutions of sera on filter paper. It was thus demonstrated that the actual albumin numbers should read 10, 7·5, 5·2, 3·2 and 1·5.

The method has been checked by using protein solutions of known composition. Good duplication has been obtained with repeated samples of sera.

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Non-Protein Nitrogen Fractions of the Flesh of Lobsters and Crabs. By DOROTHY FRASER, W. O. KERMACK, H. LEES and J. D. WOOD. (*Department of Biological Chemistry, Aberdeen University*)

The muscles of the lobster (*Homarus vulgaris*) and the crab (*Cancer pagurus*) have been subjected to 16 hr. extraction with 80 % ethanol at room temperature. The extracts, after clarification by centrifuging, were analysed for total nitrogen, amino nitrogen (Pope & Stevens, 1939), trimethylamine oxide, volatile bases, creatine and creatinine. Two-dimensional paper chromatograms (phenol/collidine) were run to identify the amino-acids present. The amino-acids were estimated by a procedure similar to that described by Fowden (1951), except that the intensity of the ninhydrin colour was determined on a Unicam diffraction-grating spectrophotometer at a wavelength corresponding to the absorption peak of the particular amino-acid being estimated.

Of the total nitrogen present in the extracts we have accounted for about 80 %, made up as follows: 60 % as amino-acid and amide nitrogen, 5 % as volatile bases, and 15 % as trimethylamine oxide. Creatine and creatinine were present at most only in traces. The principal amino-acids in both species were proline, glycine and taurine, together with glutamine and asparagine. Certain other amino-acids were present in small amounts, but no polypeptides have been detected. In so far as our results are comparable with those of Camien, Sarlet, Duchateau & Florkin (1951), the qualitative agreement is good. Lobster blood was examined and found to contain only small amounts of soluble nitrogen compounds.

When lobster muscle was kept at room temperature for 24 hr. either in air or under nitrogen, and the product then extracted as just described, the total nitrogen content of extracts was considerably increased, the increase being located in the amino-acid and volatile base fractions, while the amount of trimethylamine oxide fell. Most of the glutamine appeared to have been hydrolysed to glutamic acid. On the other hand, when the lobster flesh was kept for 72 hr. at 0°, these post-mortem changes did not occur.

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A New Aspect of Ethanol Metabolism: Isolation of Ethyl-glucuronide. By I. A. KAMIL, J. N. SMITH and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*)

Bartlett & Barnet (1949) have shown that ethanol (1 g./kg.) marked with ^{14}C is largely oxidized to CO_2 in rats, 90 % being so oxidized and eliminated in 10 hr. Both carbon atoms yield CO_2 (Dontcheff, 1950). Neubauer (1901) suggested that it formed a glucuronide in rabbits, but Deichmann & Thomas (1943) have stated that 3·5 ml./kg. of ethanol do not affect the glucuronide output of rabbits.

We have now been able to prove that in rabbits ethanol is excreted to a minor extent as a glucuronide, the amount of conjugation rising with increasing dose. At 1 ml./kg. the conjugation was 0·5 % of the dose, at 2·4 ml./kg. 0·7 %, and at 4·6 ml./kg. 1·6 %, as estimated by the naphthoresorcinol method. (These doses are well below the toxic dose which is about 10–12 ml./kg.) That the colorimetric method was estimating glucuronic acid was confirmed by the isolation of β-ethyl-D-glucuronide from the urine, as the *triacetyl methyl ester*, m.p. 144° and $[\alpha]_D^{20°}$ −32·8 ($CHCl_3$). (Found: C, 49·75; H, 6·4. $C_{15}H_{22}O_{10}$ requires C, 49·7; H, 6·1 %.) It is well known that ethanol is rapidly oxidized *in vivo*, but the present results suggest that very large doses may partly overwhelm the oxidation mechanism and thus allow conjugation to take place.

The excretion of increased glucuronide after ethanol only lasts for 1 day after dosing. Preliminary results with methanol suggest that this alcohol also forms a glucuronide, but this has not been proved by isolation. After methanol there is a slight rise in apparent glucuronide output which lasts several days after dosing. Lund (1948) has shown that methanol is slowly oxidized and excreted compared with ethanol.

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Carotene Synthesis by some Naturally occurring Mutants of *Phycomyces blakesleeanus* and by *Phycomyces nitens*: Inhibition of Carotenogenesis by Streptomycin. By T. W. Goodwin and L. A. Griffiths. (*Department of Biochemistry, University of Liverpool*)

Phycomyces blakesleeanus var. *piloboloides* (+ and −strains), *P. blakesleeanus* mut. *gracilis* (+), *P. blakesleeanus* mut. *pallens* (−) and *P. nitens* (+) were examined qualitatively and quantitatively for polyenes. All produced the same polyenes, in the same relative amounts, as the parent strain (Goodwin, 1952). *Piloboloides* (−) and *pallens* (−) synthesize at the same rate the same absolute amount of β-carotene as the (−) strain of *P. blakesleeanus* (Garton, Goodwin & Lijinsky, 1951). *Piloboloides* (+) and *nitens* (+) synthesize the same amount as *P. blakesleeanus* (+). *Gracilis* (+), however, synthesizes as much as *P. blakesleeanus* (−); furthermore, during the early stages of growth synthesis occurs in the mutant at a much greater rate than in the parent. Dry wt. production by *P. nitens* (+) is only 60 % of that of *P. blakesleeanus* whilst that of all the others is the same. Lipid synthesis is very similar in all the organisms.

Streptomycin inhibits carotenogenesis in *Phycomyces* growing under normal conditions in the light, the degree of inhibition varying linearly with the concentration up to 0·05–0·075 % (w/v). Inhibition at this level is 60–70 %. Further increase in concentration of streptomycin up to 1·6 % (w/v) does not result in further inhibition. Similar inhibition is observed when mycelia are grown in the dark and when well developed mycelia are transferred to streptomycin-containing media. No effect on dry-weight production or lipogenesis was observed at any concentration, although growth rate is always reduced slightly in the early stages. All the polyenes present are affected equally by streptomycin, in contrast to the effect observed with diphenylamine (Goodwin, 1952).

As it is never possible, even with large concentrations of streptomycin, to reduce the carotene levels below about 40 % of the normal, it seems likely that the immediate precursors of carotene can be synthesized by two independent routes, one of which is unaffected by streptomycin. This possibility is being actively investigated.

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Action Pattern of Potato Phosphorylase. By J. M. Bailey and W. J. Whelan. (*Chemistry Department, University College of North Wales, Bangor*)

It has been assumed, but never proved, that the synthesis of amylose chains by potato phosphorylase occurs by the simultaneous apposition of glucose units (from glucose-1-phosphate) to the primer molecules (multi-chain hypothesis), as opposed to the successive synthesis of molecules of high molecular weight (single-chain hypothesis) as suggested by Bernfeld & Meutémédian (1948). If the assumption of multi-chain action were correct then synthesis by phosphorylase with a homogeneous primer would afford a homogeneous linear polysaccharide of known chain length. Such homogeneous primers have been prepared by Bailey, Whelan & Peat (1950), and the following observations confirm that synthesis with phosphorylase proceeds by a multi-chain mechanism. (1) With maltohexaose as primer in the polysaccharide synthesis, the wavelength of peak absorption ($\lambda_{max.}$) of the iodine-stained products increases gradually with time of incubation from 490 mμ. (chain length, 12 units calculated from phosphate liberation) to 645 mμ. (chain length > 450 units), corresponding with colour changes from brown through red and purple to blue. During phosphorolysis of the final product of synthesis, the shift in peak absorption was the exact reverse of that

observed in synthesis. (2) Increase in the concentration of the primer (at the same glucose-1-phosphate concentration) results in a corresponding shift in $\lambda_{max.}$ of the iodine stain of the final product. (3) The action pattern is not altered by changes of pH or temperature, both of which affect the mode of action of β-amylase, causing it to vary between a multi-chain and a single-chain mechanism (French, Knapp & Pazur, 1950; Bailey & Whelan, unpublished results). It should be mentioned that by electrokinetic ultrafiltration analysis (Mould & Synge, 1931) of three of our polysaccharide preparations of average chain lengths 33, 77 and 145 units, synthesized with maltohexaose as primer, Drs D. L. Mould and R. L. M. Synge have found that the preparations each have a fairly compact distribution of molecular weights around the average and are readily resolved from one another.

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The Minimum Chain Length for *Q*-Enzyme Action. By J. M. Bailey, S. Peat and W. J. Whelan. (*Chemistry Department, University College of North Wales, Bangor*)

Hobson, Whelan & Peat (1951) have indicated that the ability of *Q*-enzyme to form a ramified amylopectin-like polysaccharide from unbranched amylose-type chains is dependent upon the chain length of the substrate. For example, the enzyme failed to act on an amylose α-dextrin of average chain length 25 units. The observation is consistent with the view that the synthesis of α-1:6-branch linkages must be preceded by the scission of α-1:4-linkages. The purpose of the present investigation was to determine the minimum length of amylose chain which could serve as a substrate for *Q*-enzyme. The proven multi-chain action of phosphorylase (see preceding communication) makes possible the synthesis of homogeneous substrates of known chain length.

A digest containing potato phosphorylase, *Q*-enzyme, glucose-1-phosphate, maltohexaose (primer), and ammonium molybdate (phosphatase inhibitor, see Bailey, Thomas & Whelan, 1951), and a control digest from which *Q*-enzyme was omitted were incubated at 35°. Portions of each were withdrawn at intervals for the estimation of phosphate and intensity of iodine stain (absorption value 680 mμ.). The chain length of the synthetic polysaccharide at any given time was calculated from the mineral phosphate liberated. It was observed that *Q*-enzyme was without influence on the absorption value (680 mμ.) until the linear chains had attained a length greater than 42 units. In a second similar experiment portions of the digest were removed and treated with pure β-amylase (from soya bean) at pH 3·6. The β-amylolysis of the synthetic polysaccharides was complete only if the chain length were less than 40 units. With chains of greater length, branching occurred and the product was not completely hydrolysed by β-amylase. Finally, *Q*-enzyme was allowed to act on two linear polysaccharides (synthesized by phosphorylase) of chain length 58 and 30 respectively. *Q*-enzyme had a very slow action on the 30-unit chain, but branching of the 58-unit chain occurred at 20–25 times as great a rate.

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Increase in Liver Weight and some other Metabolic Effects Produced by Administration of ACTH to Young Guinea Pigs. 1. By M. N. BLAND, B. J. CONSTABLE, L. J. HARRIS and R. E. HUGHES. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

The original objective in these experiments was to ascertain whether the fall in concentration of ascorbic acid in the suprarenals (and possibly in the liver, and other tissues), which is known to follow the administration of ACTH, might, if continued long enough, ultimately cause a sufficient drainage of the vitamin from the body, as to increase the requirement for the vitamin, and so to hasten the onset of scurvy in deficient animals. Although the answers to the questions posed were mostly negative, some unexpected findings emerged in the course of the trials: in particular, remarkable increases were observed in the weights of the livers of the young growing guinea pigs given repeated doses of ACTH.

In a typical later experiment, guinea pigs were kept on a scorbutogenic basal ration (Sussex ground oats 65·0, bran 14·4, dried yeast 10·8, dried egg yolk 8·0, salt mixture 1·8; halibut-liver oil, 3 drops per animal per week) supplemented with 25 mg. daily of vitamin C. Intramuscular injections of 8 mg./day of ACTH (Armour) were given, in divided doses (4 times/day). Groups of the animals were killed and examined at 2-day intervals. After 10 days, the weights of the livers of the animals treated with ACTH ranged from 7·35 to 8·05 (as % of the body weight), compared with 4·95 to 5·35 for the controls given dummy injections for 10 days, and 4·31 to 5·51 for controls at the start of injection. The ACTH at the levels administered did not depress the normal gains in body weight.

This effect of ACTH in increasing liver weight was already apparent after only 2 days of dosing, and continued thereafter, day by day. The effect has been repeatedly observed in a series of six separate trials, extending over 8 months, in which a total of over 250 guinea pigs have been tested; and it is of a very high order of statistical significance.

The application of these findings to the construction of dose-response curves, comparing the effects of cortisone and ACTH fractions in quantitative biological assays, on guinea pigs and other species, will be discussed.

Increase in Liver Weight and some other Metabolic Effects Produced by Administration of ACTH to Young Guinea Pigs. 2. By R. E. HUGHES, L. J. HARRIS, B. J. CONSTABLE and M. N. BLAND. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

Under the conditions described in the preceding abstract, the injection of ACTH caused some decline in the concentration of ascorbic acid in the guinea pigs' livers, and also in their adrenals (which likewise increased progressively in weight, e.g. by +75 % after 10 days in one typical experiment); such findings were to be expected from results recorded in earlier literature. However, it was found that the total quantities of ascorbic acid in the two organs did not decrease, but were sometimes appreciably higher even than in the controls. No important effect was observed on the concentration of glutathione in the liver.

The concentration of glycogen in the livers of the animals treated with ACTH was several times higher than in the controls (in agreement with earlier reports), but this rise was insufficient in itself to account for the augmentation in liver weight induced by ACTH. (Thus in one typical experiment, the average weights of the livers, and the average concentrations therein of fat, water, protein (N × 6·25) and glycogen were 27·2 g., and 9·1, 64·9, 17 and 4·8 %, respectively, after 10 days of injection with ACTH, as compared with 17·9 g., and 11·53, 64·9, 22·6 and 2·13 %, respectively, for controls given dummy injections for 10 days, and as compared with 16·1 g., and 7·35, 67·8, 19·8 and 2·16 %, for comparable controls at the start of injection.)

In some further parallel experiments, young growing guinea pigs were kept on a natural, mixed diet (bran, oats, cabbage, mangold, hay, halibut liver oil), in place of the basal ration, and the results have been generally similar. On this diet the liver fat was much lower than on the basal ration containing egg yolk and yeast. In the conditions of this trial, on the natural diet, however, an unexpected but consistent effect of ACTH was to decrease the fat content of the liver (e.g. from average 2·12 % for dummy-injected controls, to average 0·83 % for ACTH-injected animals, after 10 days). Reference is made to other similar tests to examine the effect of variations in the composition of the basal diet on the response to ACTH.

The Influence of pH Value and of Carbon Source on the Nutritional Requirements of *Acetobacter suboxydans*. By A. N. HALL, K. S. TIWARI and T. K. WALKER. (*Faculty of Technology, University of Manchester*)

In the course of a more extended investigation of the nutrition of *Acetobacter* species, results were obtained for *A. suboxydans* A.T.C.C. 621, which presented certain differences from those which Stokes & Larsen (1945) and Underkofler, Bantz & Peterson (1943) obtained with this species.

In attempting to trace the origin of these discrepancies the effects of varying the carbon source and pH of the medium on growth requirements have been studied. The synthetic medium used was a modification of that described by Dunn, Shankman, Camien & Block (1947). Individual amino-acids and vitamins were omitted from the medium and growth was estimated in the Spekker. A decrease to one-fourth or less of the growth in the complete medium was considered to indicate the omission of an essential nutrient, whereas a fall to two-thirds or less was regarded as showing the absence of a stimulatory factor.

In media containing glycerol or sorbitol, *A. suboxydans* is more exacting at pH 5·8 than at pH 4·6. In glucose media, however, the requirements are most complex at the lower pH value. The range pH 5·0–5·3 appears to be critical in that in glucose media the alanine requirement changes from essential to stimulatory, and in glycerol media the valine requirement changes from non-essential to essential. The fact that valine is not required by the organism in glucose media, whereas it is required in glycerol media at pH 5·3 or higher, points to a function of glucose in valine synthesis not possessed by glycerol.

Similar experiments have been directed towards the vitamin requirements of the organism in the same medium. Nicotinic acid has been found to be an essential factor in glycerol media at pH 5·0 and 6·0. However, in the presence of glucose at pH 6·0 the vitamin is not essential, whereas it is essential at pH 5·0.

It is of interest that Doede (1945) has reported that nicotinic acid is essential for *Shigella paradysenteriae* at higher pH values, but not at lower values. These results appear to be the reverse of our findings for *A. suboxydans*.

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The Fluorescence of Teeth. By R. L. HARTLES and A. G. LEAVER. (*Biochemistry Department, School of Dental Surgery, University of Liverpool*)

Stübel (1911) found that when irradiated with ultraviolet light the teeth exhibited an intense blue fluorescence second only in intensity to that of the lens of the eye. Benedict (1928) noted that dentine fluoresced more than enamel and with a bluer light. He also reported that the white spot in enamel indicative of dental caries did not fluoresce and that ashing or boiling with 50 % NaOH destroyed the fluorescence. Carious enamel and dentine lose their fluorescence. Tiede & Chomse (1934) believe that the fluorescence is due to organic material, whereas Glassa & Fonda (1938) believe it to be associated with the mineral phase. The nature of the fluorescing materials may be of fundamental importance in studying the aetiology of dental caries. If the active materials prove to be organic it suggests that disintegration of the matrix occurs at a very early stage in the carious process.

On refluxing powdered dentine (containing cementum) with ethylene glycol containing 3 % KOH, we obtained a strongly fluorescent extract. The fluorescent materials were concentrated by adsorption on charcoal and subsequent elution. Paper chromatography showed that there was more than one fluorescent substance present in the eluate. Acid hydrolysis of the eluate did not destroy the fluorescence; acetylation and methylation of this hydrolysate produced ether-soluble fluorescing materials. The activity is much stronger than can be accounted for by the amino-acids present.

It is concluded, therefore, that the fluorescence of dentine and cementum is due (at least in part) to organic compounds other than amino-acids.

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Chromatographic Examination of Cod-Liver Oil. By R. A. MORTON. (*Department of Biochemistry, University of Liverpool*)

The validity of correction procedures for estimating irrelevant absorption in the determination of vitamin A may be tested by examining the ultraviolet absorption curves of fractions obtained after chromatography on slightly weakened alumina, using light petroleum for development. The earliest fractions to pass through the column show minimal irrelevant absorption in the region 260–290 mμ., but this increases with continued elution. The absorption curve from 310–410 mμ. is constant in form (on a scale where $E_{max.} = 1{\cdot}0$) and is significantly greater from 340–410 mμ. than for *all-trans*-vitamin A acetate.

Later fractions, devoid of vitamin A, show absorption maxima for conjugated di-ene (230–240 mμ.), tri-ene (270 mμ.) and tetra-ene (305 and 320 mμ.) acidic groupings in glycerides. Such absorbing materials are strongly held on the adsorbent, but are eluted by means of ether-light petroleum mixtures and finally by diethyl ether. They do not appear to be artifacts, although acids recovered from the soaps (after saponification, extraction of unsaponifiable matter and subsequent acidification) tend to absorb more strongly as a result of isomerization of non-conjugated polyethenoid acids. The absorption curve of the unsaponifiable fraction has the same form as the vitamin A ester fractions after chromatography. The difference between the observed curves and that of *all-trans*-vitamin A acetate is due to the presence of vitamin A_2 and isomeric vitamin A (e.g. neovitamin A) in variable amounts. Anhydrovitamin A is not a normal contaminant.

The $SbCl_3$ colour test (on the unsaponifiable matter) gives values which in terms of the properties of *all-trans*-vitamin A acetate indicates a lower vitamin A content than corresponds with the gross E value (on the unsap.). Although the results of the colour test support the idea that unsaponifiable extracts exhibit 'linear' irrelevant absorption in the region 310–340 mμ., this is not fully established.

Low potency fish-liver oils (1500 i.u./g.) present special problems not brought out in the examination of rich oils and concentrates. The main clues are the properties of the glycerides and the higher absorption values on the long-wave side of the absorption curve.

The Distribution of Vitamins A_1 and A_2 in Amphibia. By R. M. LOVE, F. D. COLLINS and R. A. MORTON. (*Department of Biochemistry, University of Liverpool*)

The distribution of vitamins A_1 and A_2 has been much studied. Wald (1946) came to the conclusion that fish which spawn in fresh water contain predominantly vitamin A_2 while those that spawn in salt water contain mostly vitamin A_1. There are many apparent exceptions to this and in fact a very large number of fish-liver oils contain a small proportion of vitamin A_2. Vitamin A_2 has also been found in two Amphibia, a newt (*Triturus viridescens*) (Wald, 1942) and the tadpole of the bullfrog (*Rana catesbiana*) (Wald, 1946). The adult bullfrog, however, had only vitamin A_1.

In the present work no vitamin A_2 but only vitamin A_1 has been found in tadpoles and adults of *Rana temporaria* and *R. esculenta*. Even in tadpoles treated with thiourea, which delayed metamorphosis and allowed the animals a longer period in a fresh water environment, no vitamin A_2 was found.

Vitamin A_2 was found in two species of newt (*Triton carnifex* and *T. cristata*). In the former case a detailed study has been made of the absorption spectra of the lipids obtained from the livers, fat bodies, ovaries, testes, spleens, guts and the remainder of the body. The liver contained 1·5 mg. of vitamin A_1 and 2·1 mg. of vitamin A_2 per 100 g. of tissue.

It was thought that an amphibian, the axolotl (*Amblystoma tigrinum*), which was entirely aquatic and which remained in the larval form throughout its life would offer a significant comparison with the aquatic bullfrog tadpole and the partially aquatic newt. Axolotl eggs were hatched and the young axolotls reared in the laboratory. At first they were fed *Daphnia pulex* and a water plant (*Elodea canadensis*). After 4 months they were fed chopped meat or earthworms. No vitamin A could be detected in the livers, but the animals appeared healthy. When vitamin A was given it was stored in the liver: when β-carotene was given there was no conversion to vitamin A and little liver storage of unchanged carotene.

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The Distribution of Vitamin A in the Rat-Liver Cytoplasm. By F. D. COLLINS. (*Department of Biochemistry, University of Liverpool*)

Goerner (1938) reported the presence of vitamin A in mitochondria. This has now been confirmed. A rat liver was separated into four fractions by differential centrifugation in isotonic sucrose by the procedure of Hogeboom, Schneider & Pallade (1948). The nuclear fraction contained 7·0 μg. of vitamin A. This fraction was probably contaminated with intact cells and some mitochondria (cf. Potter, Recknagel & Hurlbert, 1951). The mitochondria contained 41·0 μg. of vitamin A while the microsomes had 0·8 μg. As a Waring blender had been used, the microsomes may have been contaminated with disintegrated mitochondria (cf. Potter *et al.* 1951). Finally the soluble fraction had 156 μg. of vitamin A.

Ernster, Zetterström & Lindberg (1950) have claimed that a water-soluble form of vitamin A can be extracted with trichloroacetic acid solution from liver. An attempt to confirm this was made, but no clear-cut distinction could be made between a water-soluble form of vitamin A and a colloidal solution of lipid droplets containing vitamin A.

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DEMONSTRATION

The Fluorescence of Teeth and Tooth Extracts. By R. L. HARTLES and A. G. LEAVER. (*Biochemistry Department, School of Dental Surgery, University of Liverpool*)